

Section 1: Identification of Marijuana

I. Introduction:

Marijuana samples will be tested and analyzed by microscopic analysis, along with The Modified Duquenois Test, or The Rapid Modified Duquenois Test, as well as GC and GC/MS for samples weighing over one ounce.

II. Reagents:

A.) Modified Duquenois Test:

1. Petroleum Ether
2. Duquenois Reagent: 10 mg Vanillan, 7 mL Acetaldehyde, in 500 mL of Ethanol
3. Concentrated Hydrochloric Acid
4. Chloroform

B.) Rapid Modified Duquenois Test:

1. Duquenois Reagent: 10 mg Vanillan, 7 mL Acetaldehyde, in 500 mL of Ethanol.
2. Concentrated Hydrochloric Acid
3. Chloroform

C.) Chromatography by GC and GC/MS:

1. Petroleum Ether
2. Methanol: used for solvent rinse on the instrument

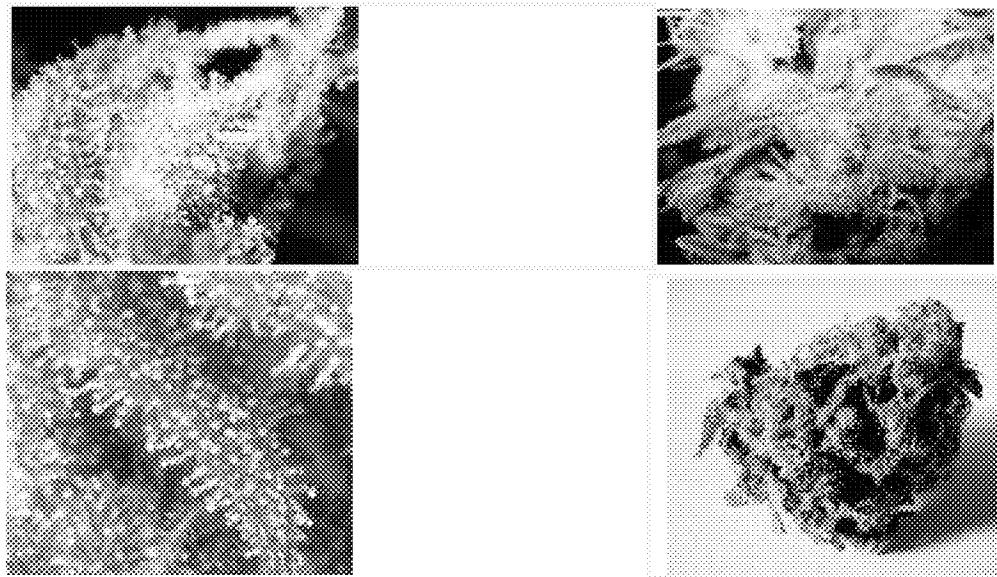
III. Equipment:

- A.) Analytical Balance
- B.) Microscope and slides
- C.) Test Tubes
- D.) Hot Plate (for when samples are moist)
- E.) Porcelain Dish
- F.) 2 mL Autosampler Vials with Teflon caps
- G.) 10 mL Volumetric flask
- H.) GC/FID: HP 6890 or 7890A
- I.) GC/MS: HP 6890/5973 or HP 7890A/5975C Series.

IV. Procedure:

A.) Microscopic Test:

1. Open sample bag and remove portion of sample and place on slide.
2. Identify characteristic morphological (both macroscopic and microscopic) features of Cannabis sativa by concentrating on leaves, small twigs, seed hulls, cystolith hairs, glandular hairs, and flowering tops.
3. Both cystolith hairs and glandular hairs should be observed to be considered positive.



B.) Modified Duquenois Test:

1. Extract 30-100 mg of sample with 15-20 mL of Petroleum Ether
2. Filter and evaporate the filtrate in a white porcelain dish.
3. Add 2mL of Duquenois reagent and stir to bring residue into solution.
4. Add 2 mL of concentrated Hydrochloric Acid, stir and let stand 10 minutes. A color will develop.
5. Transfer colored solution to labeled test tube and shake with 1-2 mL of Chloroform.
6. If marijuana is present in the sample, the violet color will be transferred to the Chloroform layer.

C.) Rapid Modified Duquenois Test:

1. Place 25-60 mg of dry crushed marijuana in a test tube and shake with 2 mL of Duquenois reagent for 1 minute.
2. Add an equal amount of concentrated Hydrochloric Acid and observe the color changes to a final violet shade.
3. Shake the mixture with 1-2 mL Chloroform.
4. If marijuana is present in the sample the violet shade will be transferred to the Chloroform layer.

D.) Residues and Smoking Apparatus's (SMAPPS):

1. Solvent rinse the apparatus containing sample or residue with Petroleum Ether.
2. Evaporate off the solvent to about 1-2 mL.
3. Transfer this solvent to a 2 mL autosampler vial to be run on GC/MS.
4. Evaporate off any leftover solvent until dry.
5. Proceed with the Modified Duquenois Test (IV.B or IV.C)

E.) Chromatography by GC and GC/MS:

1. For marijuana samples that are over 1 ounce, residues and smoking apparatus's, samples will be run on GC followed by GC/MS for confirmation.
2. Add between 30-100 mg of sample to an autosampler vial, enough so the bottom of the vial is covered.
3. Add 1-2 mL of Petroleum Ether to the autosampler vial, cap and shake a few times.
4. Place vial(s) on the GC/FID autosampler and run with the following sequence: Standard, Blank, Samples.
5. GC/FID conditions are as follows:

Method: EXP.M

Oven:

Initial Temp: 245°C

Initial Time: 0.00 min.

Rate: 10°/min.

Final Temp: 290°C

Run Time: 10 min.

Max. Temp: 325°C

Equilibration Time: 0.5 min.

Inlet:

Mode: split (35:1)
Initial Temp: 250°C
Pressure: 24.99 psi
Gas Type: Helium
Column:

Capillary: HP-1 30m x 320um

Initial Flow: 3.3 mL/min.

Detector:

Temp: 300°C
Hydrogen Flow: 30.0 mL/min.
Air Flow: 400 mL/min.
Makeup Gas: Helium

6. Obtain chromatographs. If cocaine is present, the instrument will detect a peak with a retention time around 3.40 minutes and will generate a report with accompanying chromatograph.
7. Check concentration to determine if dilutions are needed or if the injection volume needs to be increased for subsequent GC/MS run. Also observe any erroneous data that indicates that the sample may have to be reinjected.
8. Place same sequence on the GC/MS autosampler and run.
9. GC/MS conditions:

Method: EXP
Oven:
Initial Temp: 230°C
Initial Time: 0.00 min.
Max. Temp: 325°C
Equilibrium Time: 0.50 min.
Rate: 10.0°/sec
Final Temp: 280°C
Run Time: 10.00 min.

Inlet:

Injector Temp: 250°C
Mode: Split
Pressure: 31.65 psi
Gas Type: Helium
Column:
Initial Flow: 1.0 mL/min.
Column: Capillary (30m long, 320 um diameter) HP1MS

10. If THC is present in the sample, the GC/MS will detect the peak for delta-9-THC, and will generate a report with chromatograph. delta-9-THC data is stored in the GC/MS

library and is used to confirm hits detected on the GC/MS. The library can also be accessed directly without needing to run a sample first. (See graph, last page).

E.) THC Quantitation Procedure:

1. Make Stock Solution: $C_{30}H_{64}$, 2 mg/mL in Petroleum Ether
2. Make Working Solution by transferring 5 mL of stock solution to a 10 mL volumetric flask, add 1 mL THC standard (10 mg/mL in Ethanol), and bring to volume with Petroleum Ether.
3. Prepare sample by measuring 400 mg of sample and soaking in Petroleum Ether overnight. Next, filter and evaporate to about 3 mL (do not let go dry). In a 10 mL volumetric flask, add 5 mL of stock solution, 3 mL of sample, then rinse container containing the sample with Petroleum Ether to bring volume volumetric flask to volume.
4. Transfer solutions mentioned above to appropriately labeled autosampler vials and run on GC. The sequence run is as follows: THC STD., THC STD (Calib.), THC STD., BLK, SAMPLE (S), THC STD.
5. Calculations for % THC:

$$\frac{\text{PK HT THC(sample)} \times \text{PK HT}(C_{30}H_{64} \text{ STD}) \times \text{mg THC(STD)}}{\text{sample(mL)} \times 100\%} \times \frac{\text{tot.vol}}{\text{PK HT}(C_{30}H_{64}) \text{ wt.sample (mg)}} \times \frac{\text{mL(STD)}}{\text{PK HT THC(STD)}}$$

V. Results:

- A.) For the Duquenois Tests, record all results, including date, sample number, and results on the Drug Lab Results sheet that are with samples.
- B.) The results from the Drug Lab Results sheets will then be reported to the submitting agency on formal Certificate of Analysis.
- C.) When performing analysis on GC and GC/MS, print out results with chromatographs, and report as stated above. File chromatographs so they may be accessed if necessary.

